We claim:

1. A polypeptide for monitoring a change of a molecular environment associated with contact of at least two different compartments, said polypeptide comprising a plurality of amino acid sequences wherein at least one of said amino acid sequences of said polypetide targets said polypeptide to one compartment and wherein at least one other amino acid sequence of said polypeptide participates in the generation of an optical signal upon contact of the polypeptide with a second compartment.

2. The polypeptide according to claim 1 wherein said targeting amino acid sequence targets said polypeptide to exocytotic vesicle membranes and wherein the amino acid sequence which participates in the generation of the optical signal is located at the lumenal surface of said vesicle membranes.

3. The polypeptide according to claim 2 wherein said targeting amino acid sequence targets said polypeptide to synaptic vesicle membranes.

4. The polypeptide according to claim 3 wherein said targeting amino acid sequence is selected from the group consisting of synaptic vesicle proteins.

5. The polypeptide according to claim 4 wherein the said targeting amino acid sequence is selected from the group consisting of synaptotagmin and VAMP/synaptobrevin.

6. The polypeptide according to claim 1 wherein the amino acid sequence which participates in the generation of the optical signal is selected from the group consisting of a) amino acid sequences which contain a pH sensitive chromophore or flourophore, b) self quenching fluorescent amino acid sequences which fluoresce upon dilution into the extracellular space, c) enzymatic sequences which react with a fluorogenic substrate present in the second compartment, and d) amino acid sequences which bind to a specific immunological reagent present in the

second compartment.

7. The polypeptide according to claim 6 wherein the amino acid sequence which participates in the generation of the optical signal comprises an amino acid sequence which reacts with a fluorogenic substrate present in the extracellular space.

8. The polypeptide according to claim 6 wherein the amino acid sequence possesses luciferase activity or is a pH sensitive GFP.

9. The polypeptide according to claim 8 wherein the amino acid sequence is selected from the group consisting of a luciferase, an amino acid sequence sufficiently homologous to a luciferase to maintain luciferase activity, a ratiometric pHluorin, and an ecliptic pHluorin.

10. The polypeptide according to claim 9 wherein the amino acid sequence is *Cypridina* luciferase.

11. The polypeptide according to claim 1 wherein the targeting amino acid sequence targets said polypeptide to synaptic vesicles and wherein the second compartment is the extracellular space.

12. The polypeptide according to claim 11 wherein the amino acid sequence which participates in the generation of the optical signal possesses luciferase activity or is a pH sensitive GFP.

13. The polypeptide according to claim 12 wherein the targeting amino acid sequence is selected from the group consisting of synaptotagmin and VAMP/synaptobrevin and the amino acid sequence which participates in the generation of the optical signal is selected from the group consisting of a luciferase, an amino acid sequence sufficiently homologous to a luciferase to

maintain luciferase activity. 8F3 GFP. C6 GFP, S202H GFP. 1B11 GFP and 14E12 GFP.

14. The polypeptide according to claim 13 wherein the targeting amino acid sequence is selected from the group consisting of synaptotagmin and VAMP/synaptobrevin and the amino acid sequence which participates in the generation of the optical signal is 8F3 GFP or C6 GFP.

15. A nucleic acid sequence encoding a polypeptide for monitoring a change of a molecular environment associated with contact of at least two different compartments, said polypeptide comprising a plurality of amino acid sequences wherein at least one of said amino acid sequences of said polypetide targets said polypeptide to one compartment and wherein at least one other amino acid sequence of said polypeptide participates in the generation of an optical signal upon contact of the polypeptide with a second compartment.

16. The nucleic acid sequence according to claim 15 wherein said targeting amino acid sequence targets said polypeptide to exocytotic vesicle membranes and wherein the amino acid sequence which participates in the generation of the optical signal is located at the lumenal surface of said vesicle membranes.

17. The nucleic acid sequence according to claim 16 wherein said targeting amino acid sequence targets said polypeptide to synaptic vesicle membranes.

18. The nucleic acid sequence according to claim 17 wherein said targeting amino acid sequence is selected from the group consisting of synaptic vesicle proteins.

19. The nucleic acid sequence according to claim 18 wherein said

targeting amino acid sequence is selected from the group consisting of synaptotagmin and VAMP/synaptobrevin.

20. The nucleic acid sequence according to claim 15 wherein the amino acid sequence required for generation of the optical signal is selected from the group consisting of a) amino acid sequences which contain a pH sensitive chromophore or fluorophore, b) self-quenching fluorescent amino acid sequences which fluoresce upon dilution into the extracellular space, c) enzymatic sequences which react with a fluorogenic substrate present in the second compartment, and d) amino acid sequences which bind to a specific immunological reagent present in the second compartment.

21. The nucleic acid according to claim 20 wherein the amino acid sequence which participates in the generation of the optical signal comprises an amino acid sequence which reacts with a fluorogenic substrate present in the second compartment which is extracellular space.

22. The nucleic acid sequence according to claim 20 wherein the amino acid sequence possesses luciferase activity or is a pH sensitive GFP.

23. The nucleic acid sequence according to claim 22 wherein the amino acid sequence is selected from the group consisting of a luciferase, an amino acid sequence sufficiently homologous to a luciferase to maintain luciferase activity, S202H GFP, 8F3 GFP, C6 GFP, 1B11 GFP and 14E12 GFP.

24. The nucleic acid sequence according to claim 23 wherein the amino acid sequence is *Cypridina* luciferase.

25. The nucleic acid sequence according to claim 23 wherein the amino acid sequence is 8F3 GFP or C6 GFP.

26. The nucleic acid sequence according to claim 15 wherein the targeting amino acid sequence targets said polypeptide to synaptic vesicles and wherein the second compartment is the extracellular space.

27. The nucleic acid sequence according to claim 26 wherein the amino acid sequence which participates in the generation of the optical signal possesses luciferase activity or is a pH sensitive GFP.

28. The nucleic acid sequence according to claim 27 wherein the targeting amino acid sequence is selected from the group consisting of synaptotagmin and VAMP/synaptobrevin and the amino acid sequence which participates in the generation of the optical signal is selected from a luciferase, an amino acid sequence sufficiently homologous to a luciferase to maintain luciferase activity, S202H GFP, 8F3 GFP, C6 GFP, 1B11 GFP and 14E12 GFP.

29. The nucleic acid sequence according to claim 27 wherein the targeting amino acid sequence is selected from the group consisting of synaptotagmin and VAMP/synaptobrevin and the amino acid sequence which participates in the generation of the optical signal is 8F3 GFP or C6 GFP.

30. The nucleic acid sequence according to claim 15 operatively linked to a promoter sequence.

31. The nucleic acid sequence according to claim 30 wherein the promoter sequence causes expression of said protein in specific cell types.

32. The nucleic acid sequence according to claim 31 wherein the promoter sequence causes expression of said protein in neuronal cells.

33. The nucleic acid sequence according to claim 32 wherein the promoter sequence is the promoter of the immediate-early gene encoding ICP4.

34. The nucleic acid sequence according to claim 32 wherein the targeting amino acid sequence is selected from the group consisting of synaptotagmin and VAMP/synaptobrevin and the amino acid sequence which participates in the generation of the optical signal is selected from a luciferase, an amino acid sequence sufficiently homologous to a luciferase to maintain luciferase activity, S202H GFP, 8F3 GFP, C6 GFP, 1B11 GFP and 14E12 GFP.

35. The nucleic acid sequence according to claim 32 wherein the targeting amino acid sequence is selected from the group consisting of synaptotagmin and VAMP/synaptobrevin and the amino acid sequence which participates in the generation of the optical signal is 8F3 GFP or C6 GFP.

36. A plasmid comprising a nucleic acid sequence of any one of claims 30 to 35.

37. The plasmid according to claim 36 wherein the plasmid is derived from the mammalian expression vector pα4"a".

38. The plasmid according to claim 37 comprising DNA encoding a luciferase linked through its C-terminus via a linker to the N-terminus of synaptotagmin.

39. The plasmid according to claim 38 wherein the DNA encoding luciferase also encodes a cleavable signal peptide which provides for membrane translocation of the expressed protein.

40. A cell comprising a nucleic acid sequence encoding a polypeptide for monitoring a change of a molecular environment associated with contact of at least two different compartments, said polypeptide comprising a plurality of amino acid sequences wherein at least one of said amino acid sequences of said polypetide targets said polypeptide to one compartment and wherein at least one other amino

acid sequence of said polypeptide participates in the generation of an optical signal upon contact of the polypeptide with a second compartment.

41. The cell according to claim 40 wherein the compartment targeted by the targeting amino acid sequence is a mammalian cell.

42. The cell according to claim 41 wherein the mammalian cell is a neuronal cell.

43. The cell according to claim 42 wherein said targeting amino acid sequence targets said polypeptide to synaptic vesicle membranes.

44. The cell according to claim 43 wherein said targeting amino acid sequence is selected from the group consisting of synaptic vesicle proteins.

45. The cell according to claim 44 wherein the said targeting amino acid sequence is selected from the group consisting of synaptotagmin and VAMP/synaptobrevin.

46. The cell according to claim 40 wherein the amino acid sequence which participates in the generation of the optical signal is selected from the group consisting of a) amino acid sequences which contain a pH sensitive chromophore or flourophore, b) self quenching fluorescent amino acid sequences which fluoresce upon dilution into the extracellular space, c) enzymatic sequences which react with a fluorogenic substrate present in the second compartment, and d) amino acid sequences which bind to a specific immunological reagent present in the second compartment.

47. The cell according to claim 46 wherein the amino acid sequence which participates in the generation of the optical signal comprises an amino acid sequence which reacts with a fluorogenic substrate present in the second

compartment.

48. The cell according to claim 46 wherein the amino acid sequence possesses luciferase activity or is a pH sensitive GFP.

49. The cell according to claim 48 wherein the amino acid sequence is selected from a luciferase, an amino acid sequence sufficiently homologous to a luciferase to maintain luciferase activity, and a pH sensitive GFP.

50. The cell according to claim 49 wherein the amino acid sequence is *Cypridina* luciferase.

51. The cell according to claim 43 wherein the targeting amino acid sequence targets said polypeptide to synaptic vesicles and wherein the second compartment is the extracellular space.

52. The cell according to claim 51 wherein the amino acid sequence which participates in the generation of the optical signal possesses luciferase activity or is a pH sensitive GFP.

53. The cell according to claim 51 wherein the targeting amino acid sequence is selected from the group consisting of synaptotagmin and VAMP/synaptobrevin and the amino acid sequence which participates in the generation of the optical signal is selected from a luciferase, an amino acid sequence sufficiently homologous to a luciferase to maintain luciferase activity, S202H GFP, 8F3 GFP, C6 GFP, 1B11 GFP and 14E12 GFP.

54. The cell according to claim 51 wherein the targeting amino acid sequence is selected from the group consisting of synaptotagmin and VAMP/synaptobrevin and the amino acid sequence which participates in the generation of the optical signal is 8F3 GFP or C6 GFP.

55. A transgenic animal whose germ cells and somatic cells contain a nucleic acid sequence encoding a polypeptide for monitoring a change of a molecular environment associated with contact of at least two different compartments, said polypeptide comprising a plurality of amino acid sequences wherein at least one of said amino acid sequences of said polypetide targets said polypeptide to one compartment and wherein at least one other amino acid sequence of said polypeptide participates in the generation of an optical signal upon contact of the polypeptide with a second compartment.

56. The transgenic animal according to claim 55 wherein said targeting amino acid sequence targets said polypeptide to exocytotic vesicle membranes and wherein the amino acid sequence which participates in the generation of the optical signal is located at the lumenal surface of said vesicle membranes.

57. The transgenic animal according to claim 56 wherein said targeting amino acid sequence targets said polypeptide to synaptic vesicle membranes.

58. The transgenic animal according to claim 57 wherein said targeting amino acid sequence is a synaptic vesicle protein.

59. The transgenic animal according to claim 58 wherein said targeting amino acid sequence is selected from the group consisting of synaptotagmin and VAMP/synaptobrevin.

60. The transgenic animal according to claim 55 wherein the amino acid sequence which participates in the generation of the optical signal is selected from the group consisting of a) amino acid sequences which contain a pH sensitive chromophore or flourophore, b) self quenching fluorescent amino acid sequences which fluoresce upon dilution into the second compartment, c) enzymatic sequences which react with a fluorogenic substrate present in the extracellular space, and d) amino acid sequences which bind to a specific immunological reagent present in the

second compartment.

61. The transgenic animal according to claim 60 wherein the amino acid sequence which participates in the generation of the optical signal comprises an amino acid sequence which reacts with a fluorogenic substrate present in the second compartment.

62. The transgenic animal according to claim 60 wherein the amino acid sequence possesses luciferase activity or is a pH sensitive GFP.

63. The transgenic animal according to claim 62 wherein the amino acid sequence is selected from a luciferase or an amino acid sequence sufficiently homologous to a luciferase to maintain luciferase activity.

64. The transgenic animal according to claim 63 wherein the amino acid sequence is *Cypridina* luciferase.

65. The transgenic animal according to claim 51 wherein the targeting amino acid sequence targets said polypeptide to synaptic vesicles and wherein the second compartment is the extracellular space.

66. The transgenic animal according to claim 65 wherein the amino acid sequence which participates in the generation of the optical signal possesses luciferase activity or is a pH sensitive GFP.

67. The transgenic animal according to claim 66 wherein the targeting amino acid sequence is selected from the group consisting of synaptotagmin and VAMP/synaptobrevin and the amino acid sequence which participates in the generation of the optical signal is selected from a luciferase, an amino acid sequence sufficiently homologous to a luciferase to maintain luciferase activity,

S202H GFP, 8F3 GFP, C6 GFP, 1B11 GFP and 14E12 GFP.

68. The transgenic animal according to claim 66 wherein the targeting amino acid sequence is selected from the group consisting of synaptotagmin and VAMP/synaptobrevin and the amino acid sequence which participates in the generation of the optical signal is 8F3 GFP or C6 GFP.

69. The transgenic animal according to claim 55 wherein the nucleic acid sequence is operatively linked to a promoter sequence which causes expression of said protein in specific cell types.

70. The transgenic animal according to claim 69 wherein the promoter sequence causes expression of said protein in neuronal cells.

71. The transgenic animal according to claim 70 wherein the promoter sequence is the promoter of the immediate-early gene encoding ICP4.

72. The transgenic animal according to claim 71 wherein the targeting amino acid sequence is selected from the group consisting of synaptotagmin and VAMP/synaptobrevin and the amino acid sequence which participates in the generation of the optical signal is selected from a luciferase, an amino acid sequence sufficiently homologous to a luciferase to maintain luciferase activity and a pH sensitive GFP.

73. A method of detecting the release of intracellular substances comprising:

a) obtaining cells containing a polypeptide comprising a plurality of amino acid sequences wherein at least one of said amino acid sequences of said polypetide targets said polypeptide to an intracellular location and wherein at least

one other amino acid sequence of said polypeptide which participates in the generation of an optical signal upon contact of the polypetide with the environment external to the cell:

b) detecting an optical signal upon release of the polypeptide of step (a) into the environment external to the cell wherein said optical signal is generated by contact of the polypeptide with the environment external to said cell.

74. The method according to claim 73 wherein said targeting amino acid sequence targets said polypeptide to exocytotic vesicle membranes and wherein the amino acid sequence required for generation of the optical signal is located at the lumenal surface of said vesicle membranes.

75. The method according to claim 74 wherein said targeting amino acid sequence targets said polypeptide to synaptic vesicle membranes.

76. The method according to claim 75 wherein said targeting amino acid sequence is a synaptic vesicle protein.

77. The method according to claim 76 wherein the said targeting amino acid sequence is selected from the group consisting of synaptotagmin and VAMP/synaptobrevin.

78. The method according to claim 73 wherein the amino acid sequence which participates in the generation of the optical signal is selected from the group consisting of a) amino acid sequences which contain a pH sensitive chromophore or flourophore, b) self quenching fluorescent amino acid sequences which fluoresce upon dilution into the extracellular space, c) enzymatic sequences which react with a fluorogenic substrate present in the extracellular space, and d) amino acid sequences which bind to a specific immunological reagent present in the extracellular space.

79. The method according to claim 78 wherein the amino acid sequence required for generation of the optical signal comprises an amino acid sequence which reacts with a fluorogenic substrate present in the extracellular space.

80. The method according to claim 78 wherein the amino acid sequence possesses luciferase activity or a pH sensitive GFP.

81. The method according to claim 80 wherein the amino acid sequence is selected from a luciferase or an amino acid sequence sufficiently homologous to a luciferase to maintain luciferase activity.

82. The method according to claim 81 wherein the amino acid sequence is *Cypridina* luciferase.

83. The method according to claim 73 wherein the targeting amino acid sequence targets said polypeptide to synaptic vesicles and wherein the amino acid sequence which participates in the generation of the optical signal comprises an amino acid sequence which reacts with a fluorogenic substrate present in the extracellular space.

84. The method according to claim 73 wherein the amino acid sequence which participates in the generation of the optical signal possesses luciferase activity or is a pH sensitive GFP.

85. The method according to claim 84 wherein the targeting amino acid sequence is selected from the group consisting of synaptotagmin and VAMP/synaptobrevin and the amino acid sequence which participates in the generation of the optical signal is selected from a luciferase, an amino acid sequence sufficiently homologous to a luciferase to maintain luciferase activity or a pH sensitive GFP.

86. A pH sensitive mutant of GFP of *Aequora victoria* wherein a change in pH results in an alteration in one or more spectral properties, including intensity, of the excitation and/or emission spectra.

87. A pH sensitive GFP according to claim 86 wherein amino acid residues within at least one amino acid on either side of one or more amino acids selected from the group consisting of the amino acids at positions 94, 96, 148, 167, 203, 205 and 222, are altered.

88. A pH sensitive GFP according to claim 86 wherein at least one substitution is made at an amino acid position selected from the group consisting of positions 147, 149, 161, 163, 166, 167, 168, 175 and 202.

89. A pH sensitive GFP according to claim 86 comprising at least one of the mutations selected from the group consisting of S147E, S147P, N149V, N149Q, N149T, N149L, N149D, N149Y, N149W, T161I, K166Q, I167V, R168H, and S202H.

90. A pH sensitive GFP according to claim 86 comprising at least one mutation selected from the group consisting of S147D, N149Q, N149D, T161I, K166Q, I167V and S202H.

91. A pH sensitive GFP according to claim 86 comprising S147D, N149Q and T161I mutations.

92. A pH sensitive GFP according to claim 91 further comprising V163A and S175G mutations.

93. A pH sensitive GFP according to claim 86 comprising S147D, N149D, K166Q, I167V and S202H mutations.

94. A pH sensitive GFP according to claim 93 further comprising V163A and S175G mutations.

95. A pH sensitive GFP according to claim 86 wherein an attenuation or loss of the excitation peak at 475 nm and a loss of fluorescence intensity excitable at 395 nm occurs upon a decrease of pH.

96. A pH sensitive GFP according to claim 95 selected from the group consisting of 1D10, 2F10, 2H2, 1B11, 8F6, 8F3 and 19E10.

97. A pH sensitive GFP according to claim 86 which exhibit a decreased fluorescence due to excitation at the 395 nm peak and increased fluorescence due to increased excitation at the 475 nm peak in response to a decrease in pH.

98. A pH sensitive GFP according to claim 97 selected from the group consisting of 14E12, 14C9, 14C8, 2G3, S202, H14D9, C6 and 8H8.

99. A fusion protein comprising the pH sensitive GFP according to claim 86 and at least one other amino acid sequence.

100. The fusion protein according to claim 99 wherein said other amino acid sequence targets the fusion protein to a cell.

101. A nucleic acid molecule encoding a pH sensitive GFP protein of any one of claims 86 through 100.